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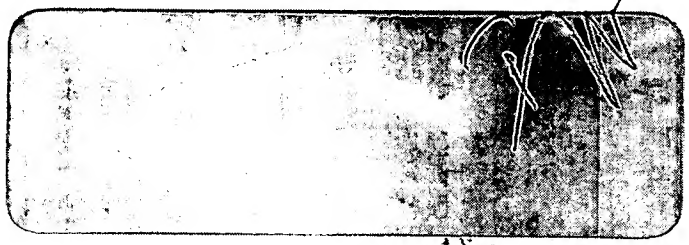

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/852,958	05/10/2001	David A. Sirbasku	1944-00201	5333

7590 03/03/2005

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EXAMINER	
UNGAR, SUSAN NMN	

ART UNIT	PAPER NUMBER
1642	

DATE MAILED: 03/03/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

DOCKET NO. Z1300-57390(04-344)
SERIAL NO./~~USPN/REG. NO.~~ 29/206,623
FILED/~~ISSUE/REG.~~ 6-1-04
APPLICANT: Smith
ASSIGNEE/~~MARK~~: Zebco

The U.S. Patent & Trademark Office acknowledges and has stamped hereon the date of receipt of items checked below which were mailed 12-13-04.

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    [ ] PROVISIONAL
    [ ] DESIGN
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Office Action Summary	Application No.	Applicant(s)	
	09/852,958	SIRBASKU, DAVID A.	
	Examiner	Art Unit	
	Susan Ungar	1642	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on *15 June 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☐ Claim(s) *4-10,12-86,95-101 and 109-122* is/are pending in the application.

4a) Of the above claim(s) *4-10,12-33,44-86,96-101 and 115-122* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) *34-43,95 and 109-114* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *9/16/2002*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

2. The Election filed June 15, 2004 in response to the Office Action of October 3, 2003 is acknowledged and has been entered. Claims 1-108 were pending in the application, claims 1-3, 11, 87-94, 102-108 have been canceled, claims 34, 38, 44, 56, 63, 95, 99 have been amended and Claims 4-10, 12-33, 44-86, 96-101, 115-122 (all of whom are dependent upon claim 44 and are therefore joined to the group comprising claim 44) have been withdrawn from further consideration by the examiner under 37 CFR 1.142(b) as being drawn to non-elected inventions. Claims 34-43, 95, 109-114 are currently under prosecution.

3. Applicant's election with traverse of Group 10, claims 34-43 is acknowledged. The traversal is on the ground(s) that Groups 10, 11, 14, 15, 19, 20, 23 and 24 are all classified in Class 435, subclass 4 and therefore search of all of the claim together would not be unduly burdensome on the Examiner. The argument has been considered but has not been found persuasive because although the classification of the groups is the same, classification of subject matter is merely one indication of the burdensome nature of the search involved. The literature search, particularly relevant in this art, is not coextensive. Different searches and issues are involved in the examination of each group.

Applicant further argues that the methods of Groups 10, 13-15, 20 share steps, techniques and materials wherein the underlying assay methods and materials are similar and therefore the examination of all of the groups would not be an undue burden. The argument has been considered but has not been found persuasive because as previously set forth, the methods are distinct because they differ at least in objectives, method steps, reagents and/or dosages and/or schedules used, response variables, and criteria for success and therefore examination of all of these group would be an undue burden.

Applicant further argues that the cell culture medium is useful in the methods of Groups 10, 11, 13, 14, 15, 19, 20, 23, 24 and is linked to the claims. The argument has been considered but has not been found persuasive, the cell culture medium is distinct and properly restricted from the claimed method for the reasons of record.

Applicant argues that the assay kits of claims 63-69 should be rejoined to Group 10 because claim 63 has been amended to require the culture medium of claim 45. The argument has been considered but has not been found persuasive because, for the reasons set forth above, the group comprising claim 45 has not been rejoined to Group 10.

Applicant reiterates argues drawn to Group 13, claim 56 and argues that claim 56 also provides for reversal of inhibition of thyroid hormone responsive cells by thyroid hormone. The arguments have been considered but have not been found persuasive for the reasons set forth above and further because the claim does not provide for reversal of inhibition by thyroid hormone but rather provides for optionally adding an inhibition reversing amount of thyroid hormone.

Applicant requests that the restriction requirement be redrawn in the light of the foregoing arguments and amendments. In light of the foregoing arguments and amendments, claim 95 has been rejoined with Group 10 and claims 109-114 as set forth above have been joined with Group 10. It is further noted that although claim 95 has been rejoined to Group 10, due to the amendment of the claim. However, claims 96-98 have not been rejoined because they are drawn to materially distinct methods which differ at least in objectives, method steps, reagents and/or dosages and/or schedules used, response variables, and criteria for success from the method originally claimed and therefore are withdrawn from consideration and only the

invention constructively elected by original presentation for prosecution on the merits will be examined.

However, the remaining arguments have been considered but have not been found persuasive. For these reasons the restriction requirement is deemed to be proper and is therefore made FINAL.

Applicant notes that in the Office Action of October 3, 2003 there is no Group 8 and claim 62 has not been assigned to a restriction group. Examiner apologizes for the inadvertent typographical error and hereby assigns claim 62 to Group 25.

Group 25 is drawn to a method of propagating a virus in the medium of claim 45 classified in Class 435, subclass 235.1. This Group is distinct from all of the other method groups because it is a materially distinct method which differs at least in objectives, method steps, reagents and/or dosages and/or schedules used, response variables, and criteria for success.

The inventions of Groups 12 and 25 are related as product and process of use. The inventions can be shown to be distinct if either or both of the following can be shown: (i) the process for using the product as claimed can be practiced with another materially different product or (ii) the product as claimed can be used in a materially different process of using that product [see *MPEP* § **806.05(h)**]. In the instant case the cell culture medium as claimed can be used in a materially different process such as culturing hormone-responsive cell lines.

Given that Applicant has elected an invention for examination, given that Applicant has not traversed the lack of assignment of claim 62 to a restrictive group or requested the rejoined of claim 62 to the elected group, given that Examiner has assigned claim 62 to a specific Group and has explained the reasons

why the group is distinct, the omission of assignment of claim 62 has been remedied and Group 10 will be examined as set forth above.

4. This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR 1.8821 (a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 CFR 1.821 through 1.825 for the reasons(s) set forth on the attached Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence And/Or Amino Acid Sequence Disclosures.

Applicant is given the period of reply for the instant paper within which to comply with the sequence rules, 37 CFR 1.821-1.825. Failure to comply with these requirements will result in ABANDONMENT of the application under 37 CFR 1.821 (g). Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a). In no case may an applicant extend the period for response beyond the SIX MONTH statutory period. Direct the response to the undersigned. Applicant is requested to return a copy of the attached Notice to Comply with the response.

In particular, figures 87 and 96 are drawn to unidentified sequences. Further, on page 15, para 0028 and page 150, para 0544, the specification discloses a special inhibitor motif of six amino acids which also does not comply with the sequence rules. Examiner has made an effort to identify these informalities but applicant must carefully review the specification to identify and indicate where other unidentified sequences may be found. Appropriate correction is required.

Specification

5. The specification on page 1 should be amended to reflect the status of the parent applications. Appropriate correction is required.

6. The specification is objected to because the Table of Contents recites paragraph numbers. These numbers must be deleted since they do not correspond to the paragraph numbers of the printed application and would not be expected to correspond to the paragraph numbers of an issued patent, if such patent were issued. Appropriate correction is required.

7. The specification is objected to because there are numerous instances of blank spaces wherein ATCC accession numbers and dates are missing from the disclosure, for example, see page 44, para 024. Further, there are numerous instances of blank spaces wherein US Application numbers and PCT numbers are missing from the disclosure, for example see p. 144, para 0528. Applicant is required to either amend the specification to include the information left out of the specification or to amend the specification to delete reference to the information. Appropriate correction is required.

8. The specification is replete with instances of grammatical and/or typographical errors, for example on page 73, para 0318 "culture medium acts is a weak estrogen", page 129, para 0481 "an even more an important consequence", page 129, para 0482). Examiner has made an effort to identify these informalities but Applicant must carefully review the specification to identify and indicate where these informalities can be found. Appropriate correction is required.

Trademarks

9. The use of the numerous trademarks has been noted in this application Examples where trademarks are found are on p. 19, paragraph 0047, p. 55, paragraph 0275. However, the instances of trademarks are too numerous to detail here. Examiner has made an effort to identify these informalities but applicant must carefully review the specification to identify and indicate where recitations of

trademarks can be found. The trademarks be capitalized wherever they appear and be accompanied by the generic terminology.

Although the use of trademarks is permissible in patent applications, the proprietary nature of the marks should be respected and every effort made to prevent their use in any manner which might adversely affect their validity as trademarks.

Claim Rejections - 35 USC § 112

10. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

11. Claims 34-43, 95, 109-114 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 34-43, 95, 109-114 are indefinite because claim 34 is drawn to detecting steroid hormone-like cancer cell growth stimulation by a substance of interest. The claim is indefinite because the phrase "steroid hormone-like" is a relative phrase which renders the claim indefinite. The phrase "steroid hormone-like" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. In particular, as drawn to claim 95, since the specification, in paragraph 0052 of the published application, defines estrogenic as estrogen-like stimulation of cell proliferation, claim 95 is also

indefinite for the reasons set forth above drawn to "steroid hormone-like". Further, it is noted that US Patent No. 5,135,849 specifically teaches that the term "androgenic" has no well defined meaning in the art and is conventionally used inconsistently and indiscriminately (col 7, lines 18-30) and that US Patent No. 4,859,585 specifically teaches that the term "estrogenic" has no well defined meaning in the art and is conventionally used inconsistently and indiscriminately (col 7, lines 35-45). Thus the art recognizes that phrases drawn to "estrogenic" androgenic" and by extension "steroid hormone-like" are indefinite.

Claims 34-43, 95, 109-114 are indefinite because claim 34 comprises the limitation "substantially devoid of unbound Fe(III). The claim is indefinite because the phrase "substantially devoid" is a relative phrase which renders the claim indefinite. The "substantially devoid" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention.

12. If Applicant were able to overcome the rejection set forth above, Claims 34-39, 41-43, 95, 109-114 would still be rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of detecting steroid hormone-like cancer growth stimulation by a subject of interest comprising maintaining a predetermined population of steroid hormone-responsive cells in a steroid hormone-free nutrient medium comprising a basal nutrient fluid substantially devoid of unbound Fe(III) and containing calcium ion and a quantity of immunoglobulin inhibitor sufficient to inhibit cell growth in the absence of an inhibition-reversing amount of steroid hormone wherein said immunoglobulin inhibitor is selected from the group consisting of dimeric/polymeric serum IgA,

polymeric IgM and IgG1 kappa or a combination thereof wherein a substance of interest is added and cell population is determined, does not does not reasonably provide enablement for a method of detecting steroid hormone-like cancer growth stimulation by a subject of interest comprising maintaining a predetermined population of steroid hormone-responsive cells in a steroid hormone-free nutrient medium comprising a basal nutrient fluid substantially devoid of unbound Fe(III) and containing calcium ion and a quantity of immunoglobulin inhibitor sufficient to inhibit cell growth in the absence of an inhibition-reversing amount of steroid hormone wherein a substance of interest is added and cell population is determined. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

It is noted that in view of the indefinite nature of the claim language drawn to "steroid hormone-like cancer cell growth stimulation" in claim 34, it is assumed for examination purposes that steroid hormone-like cancer cell growth stimulation , claimed in claim 34 and the claims dependent thereon, is stimulation wherein the inhibitory action of immunological inhibitors of cell growth in steroid hormone-responsive cells is reversed.

The claims are drawn to a method of detecting steroid hormone-like cancer growth stimulation by a subject of interest comprising maintaining a predetermined population of steroid hormone-responsive cells in a steroid hormone-free nutrient medium comprising a basal nutrient fluid substantially devoid of unbound Fe(III) and containing calcium ion and comprising a quantity of immunoglobulin inhibitor sufficient to inhibit cell growth in the absence of an inhibition-reversing amount of

steroid hormone. This means any immunoglobulin inhibitor sufficient to inhibit cell growth in the absence of an inhibition-reversing amount of steroid hormone.

The specification teaches that the instant specification satisfies the long-felt needs for a sensitive way to screen substances for estrogenic and androgenic effects (page 15, para 0030). The specification teaches that since the early 1980's researchers have unsuccessfully tried to identify serum-borne inhibitors of steroid responsive cell growth and despite its first proposal more than fifteen years ago, the purified steroid reversible serum-borne inhibitor had not been previously described (pgs 2-4). Although patents have been issued drawn to a serum mediator that is a steroid reversible inhibitor of steroid responsive cell growth, the US Patents do not address the issues of whether there are one or more or disclose the exact chemical composition of the inhibitors (p. 6, para 0010).

The specification teaches that for the first time it is disclosed that, surprisingly, certain immunoglobulins exert a steroid hormone reversible negative regulatory effect on cancer cell growth (p. 16, para 0032). These immunoglobulin inhibitors have many immediate and potential applications as reagents for cell growth assays. For example, they are useful for *in vitro* testing of substances for estrogenic effects (or other steroid hormone-like effects) on steroid hormone responsive cell growth in a suitable assay system (p. 17, para 0039) and thus are useful for assaying agents of interest, such as drugs or environmental chemicals, for their steroid hormone-like effects on cell growth stimulation (p. 18, para 0042) as an aid to avoiding undesirable proliferative side effects of such drugs or substance *in vivo* (para bridging pages 19-20). The claimed method is useful for identifying substances that have unrecognized hormone-like properties that present health hazards (p. 36, para 0229).

As drawn to the broadly claimed immunoglobulin inhibitors, the specification teaches that IgA, IgM and certain IgGs provide negative regulation of steroid hormone responsive mucosal epithelial cancer cell growth (p. 15, para 0030) and for the first time it is disclosed, surprisingly, that certain immunoblogulins exert a steroid hormone reversible negative regulatory (inhibitory) effect on steroid responsive cancer growth. In the most preferred embodiments, the inhibitors is/are dimeric IgA (non-sIgA, polymeric IgM, IgG1 kappa and IgG2 (p. 16, para 0032). The specification exemplifies the chemical and immunological properties of the partially purified CA-PS-pool II of steroid hormone reversible inhibitors of cancer cell growth wherein the long sought after serum-borne cancer cell growth inhibitors were found to include at least IgA and IgM in Example 20, p. 124-129. The specification teaches that the series of investigations presented in the example demonstrate that a very longstanding problem has been solved (p. 129, para 0480), that is inhibitors have been identified. The specification teaches that dimeric/ polymeric plasma-derived IgA, but not serum monomeric IgA or sIgA, is a steroid hormone reversible inhibitor of steroid responsive cancer cell growth (p. 129, para 0481). The specification further teaches that plasma-derived multimeric IgM is a steroid hormone reversible inhibitor of steroid responsive cancer cell growth (p. 126, para 0472). The specification concludes the discussion of Example 20 and states that "This series of investigations demonstrate at least two immunoglobuin inhibitors in serum". There may still be other useful estrogen reversible inhibitors in serum that are yet to be identified from serum or tissue sources. The methods described in this Example have direct application to the search for new compounds that mimic the effect of the immunoglobulins as" steroid reversible inhibitors. (p. 130, para

0484). The specification teaches that a poly-Ig receptor or a poly-Ig like receptor mediates the inhibition of cell growth by IgA and IgM (p. 139, para 0514) but does not teach which poly-Ig receptor or poly-Ig like receptor could be used as target for development of compounds that mimic the immune system inhibition of cancer cell growth (pgs. 141-143).

The specification teaches that bulk purified mixtures of all subclasses of horse and rat IgG are not steroid hormone reversible inhibitors of steroid responsive cancer cell growth (p. 137, para 0510), but that additional studies demonstrated that IgG1 kappa alone was a significant steroid hormone reversible inhibitor of steroid responsive cancer cell growth. Although experiments with prostate cancer cells lines showed some steroid hormone reversible inhibition of steroid responsive cancer cell with IgG2 kappa the data was not shown (p. 138, para 0511) and it does not appear that this effect was significant as the discussion of Example 23 did not even mention IgG2 kappa. This discussion however, clearly discloses that the preference for the kappa light chain implies that a different receptor mediates the effects of this immunoglobulin as compared to IgA and IgM (p. 138 para 0512) and concludes that once it is identified, the receptor that mediates the IgG1 growth inhibition effect will provide another target for development of compounds that mimic the immune system inhibition of cancer cell growth (p. 138, para 0512).

One cannot extrapolate the teaching of the specification to the enablement of the claims because the specification clearly and repeatedly teaches that the identification of the immunoglobulin inhibitors was a "surprising" that is, an unexpected event and that fifteen years of combined research by those skilled in the art had failed to identify serum-borne inhibitors of steroid responsive cell

growth and despite its first proposal more than fifteen years ago, the purified steroid reversible serum-borne inhibitors had not been previously described. Further, although patents have been issued drawn to a serum mediator that is a steroid reversible inhibitor of steroid responsive cell growth, the US Patents do not address the issues of whether there are one or more or disclose the exact chemical composition of the inhibitors. However, the instant specification only sets forth three immunoglobulin inhibitors that in fact are effective as the steroid reversible immunoglobulin inhibitors of steroid-responsive cell growth. The identification of these three factors does not predictably enable the claimed invention because the specification does not teach how to make the claimed invention. Although it appears that the binding of the inhibitory immunoglobulins is through the Fc portion of the immunoglobulins, there is no teaching of which structures of the Fc portions are required for the invention to function as claimed. The specification clearly teaches that the preference for the kappa light chain of IgG implies that a different receptor mediates the effects of this immunoglobulin as compared to IgA and IgM. The specification further teaches that **a** (emphasis added) pIgR is the target for IgA and IgM but does not identify which pIgR is the target for steroid hormone reversible inhibition. However, the specification provides no information as to structures that are common to the exemplified inhibitors that would allow one of skill to predictably make the claimed inhibitors based on a structure/function correlation. Although the specification clearly teaches that once the receptors are identified, the receptors will provide another target for the development of compounds that mimic the immune system inhibition of cancer cell growth, there is no guidance as to the receptors to which the inhibitory immunoglobulins bind so that one could make inhibitors that would predictably mimic the immune system

inhibition of cancer ell growth. Finally, although the specification teaches that there may still be other useful steroid reversible inhibitors in serum that are yet to be identified from serum or tissue sources, the specification provides no guidance as to how one would predictably identify these other useful steroid reversible inhibitors.

The specification does not teach how to extrapolate the teaching of the three identified inhibitors to the broadly claimed invention because the specification does not provide information that could be used to predictably distinguish tissue inhibitory immunoglobulins from those that are not inhibitory and the screening assays exemplified in the specification for identification and testing of inhibitory immunoglobulins do not meet the standard of 35 USC 112, first paragraph because they do not teach how to make the claimed invention.

Applicant is reminded that 35 USC 112, first paragraph does not require that the specification teach how to screen for inhibitors, but rather requires that the specification teach how to make and use the claimed invention. In particular, screening assays do not enable the claimed invention because the court found in (*Rochester v. Searle*, 358 F.3d 916, Fed Cir., 2004) that screening assays are not sufficient to enable an invention because they are merely a wish or plan for obtaining the claimed chemical invention. It is clear that in the absence of an effective steroid reversible inhibitor, one would not be able to successfully use the claimed broadly claimed invention.

Given that the specification specifically teaches that the combined research of those of skill was not successful in identifying the claimed immunological inhibitors despite 15 years of research, given that the specification teaches the surprising nature of the claimed inhibitors, given that the specification teaches that

the receptors to which the immunological inhibitors bind are unknown, given that the specification does not teach, other than through screening assays how to identify the claimed invention, since the structures involved with the binding of the inhibitors to their cognate receptors are unknown, it is clear that the specification does provide the necessary guidance to one of skill to enable the making of the claimed invention and if the making of the broadly claimed invention is not enabled, one would not know how to use the broadly claimed invention.

The specification provides no working examples which would provide guidance to one skilled in the art and no evidence has been provided which would allow one of skill in the art to predictably make the claimed invention so that it would function as claimed with a reasonable expectation of success. In the absence of guidance or exemplification so that the broadly claimed immunological inhibitors could be predictably made, the screening assays taught are drawn only to a wish or a plan for making the claimed invention. For the above reasons, it appears that undue experimentation would be required to enable one to practice the claimed invention.

13. Claims 34-39, 41-43, 95, 109-114 are rejected under 35 USC 112, first paragraph, as lacking an adequate written description in the specification.

Claims 34- 39, 41-43, 95, 109-114 are drawn to steroid reversible inhibitors of steroid hormone-responsive cell growth. Although drawn to DNA arts, the findings in University of California v. Eli Lilly and Co., 119 F.3d 1559, 43 USPQ2d 1398 (Fed. Cir. 1997) and Enzo Biochem, Inc. V. Gen-Probe Inc. are relevant to the instant claims. The Federal Circuit addressed the application of the written description requirement to DNA-related inventions in University of California v. Eli Lilly and Co., 119 F.3d 1559, 43 USPQ2d 1398 (Fed. Cir. 1997).

The court stated that "[a] written description of an invention involving a chemical genus, like a description of a chemical species, requires a precise definition, such as by structure, formula, [or] chemical name,of the claimed subject matter sufficient to distinguish it from other materials. Id. At 1567, 43 USPQ2d at 1405. The court also stated that

> a generic statement such as "vertebrate insulin cDNA" or "mammalian insulin cDNA without more, is not an adequate written description of the genus because it does not distinguish the genus from others, except by function. It does not specifically define any of the genes that fall within its definition. It does not define any structural features commonly possessed by members of the genus that distinguish them from others. One skilled in the art therefore cannot, as one can do with a fully described genus, visualize or recognize the identity of the members of the genus. A definition by function, as we have previously indicated, does not suffice to define the genus because it is only an indication of what the gene does, rather than what it is.

Id. At 1568, 43 USPQ2d at 1406. The court concluded that "naming a type of material generally known to exist, in the absence of knowledge as to what that material consists of, is not a description of that material. Id.

Finally, the court addressed the manner by which a genus of cDNAs might be described. "A description of a genus of cDNAs may be achieved by means of a recitation of a representative number of cDNAs, defined by nucleotide sequence, falling within the scope of the genus or of a recitation of structural features common to the members of the genus, which features constitute a substantial portion of the genus. Id.

The Federal Circuit has recently clarified that a DNA molecule can be adequately described without disclosing its complete structure. See Enzo Biochem, Inc. V. Gen-Probe Inc., 296 F.3d 1316, 63 USPQ2d 1609 (Fed. Cir. 2002). The Enzo court adopted the standard that "the written description

requirement can be met by 'show[ing] that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics ....i.e., complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics. " Id. At 1324, 63 USPQ2d at 1613 (emphasis omitted, bracketed material in original).

The inventions at issue in Lilly and Enzo were DNA constructs per se, the holdings of those cases are also applicable to claims such as those at issue here. A disclosure that does not adequately describe a product itself logically cannot adequately describe a method of using that product.

Thus, the instant specification may provide an adequate written description of steroid reversible inhibitors of steroid hormone-responsive cell growth, per Lilly by structurally describing a representative number of steroid reversible inhibitors of steroid hormone-responsive cell growth or by describing "structural features common to the members of the genus, which features constitute a substantial portion of the genus. Alternatively, per Enzo, the specification can show that the claimed invention is complete "by disclosure of sufficiently detailed, relevant identifying characteristics, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics.

In this case, the specification does not describe the steroid reversible inhibitors of steroid hormone-responsive cell growth required to practice the claimed method in a manner that satisfies either the Lilly or Enzo standards. The IgG1 kappa inhibitor disclosed does not appear to share a relevant structure with polymeric/multimeric IgA or multimeric IgM. In addition, given the surprising

nature of the discovery, the combination of the polymeric/multimeric IgA and the multimeric IgM do not provide a representative number of immunological inhibitors that would meet the requirements of Lilly. Further, the specification does not provide any functional characteristics coupled with a known or disclosed correlation between structure and function as drawn to the inhibitory activity. Although the specification discloses three effective immunological inhibitors, this does not provide a description of the steroid reversible inhibitors of steroid hormone-responsive cell growth that would satisfy the standard set out in Enzo, or describe "structural features common to the members of the genus, which features constitute a substantial portion of the genus which would satisfy the standard set out in Lilly.

Since the specification fails to adequately describe the broadly claimed steroid reversible inhibitors of steroid hormone responsive cell growth, it also fails to adequately describe the assay method of using the claiming invention.

14. In the interests of compact prosecution, if Applicant were to amend the claims to recite further clarify the claimed inhibitors, for example to recite the limitation "comprising a steroid hormone reversible inhibitor, wherein said inhibitor consists of an immunoglobulin or a combination of immunoglobulins", and if Applicant were able to overcome the rejections under 35 USC 112, first and second paragraph above, Claims 34-43, 95, 109-113 would still be rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for the claimed method of detecting steroid hormone-like cancer growth stimulation by a subject of interest comprising maintaining a predetermined population of steroid hormone-responsive cells in a defined steroid free nutrient medium comprising 1.0 ng/mL to 10 ng/mL insulin, 0.3 - 10 nM triiodothyronine, 2 - 50

ug/mL- diferric transferrin, 5 - 100 gM elhanolamine, 0.2 - 5.0 mg/mL- bovine serum albumin (BSA), 5 - 20 ng/mL - selenium, 2 - 10 MM deferoxamine, and depending upon the requirements of the selected cells to be cultured, at least one compound chosen from the group consisting of 1 - 50 ng/mL EGF, 0.2 - 20 ng/mL - aFGF, 5 - 50 MM phosphoethanolamine, 50 - 500 ug/mL linoleic acid-BSA, 1 - 50 ug/mL reduced glutathione, 0.5 - 2.0 mM glutamine, 1 - 10 ug/mL heparin, and 20 - 50 ug human fibronectin (per 35-mm diameter culture dish) wherein the nutrient medium further comprises no more than about 1 uM unbound Fe(III) and contains about 1-50 mM calcium ion does not reasonably provide enablement for the claimed method of detecting steroid hormone-like cancer growth stimulation by a subject of interest comprising maintaining a predetermined population of steroid hormone-responsive cells in a steroid free nutrient medium wherein said medium comprises a basal nutrient fluid substantially devoid of unbound Fe(III) and containing calcium ion. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

It is noted that in view of the indefinite nature of the claim language drawn to "steroid hormone-like cancer cell growth stimulation" in claim 34, it is assumed for examination purposes that steroid hormone-like cancer cell growth stimulation , claimed in claim 34 and the claims dependent thereon as well as the estrogen-like cancer growth stimulation claimed in claim 95, is stimulation wherein the inhibitory action of immunological inhibitors of cell growth in steroid hormone-responsive cells is reversed.

The claims are drawn to a method of detecting steroid hormone-like cancer growth stimulation by a subject of interest comprising maintaining a predetermined

population of steroid hormone-responsive cells in a steroid free nutrient medium wherein said medium comprises a basal nutrient fluid substantially devoid of unbound Fe(III) and containing calcium ion. This means any medium as long as it is a basal nutrient fluid substantially devoid of unbound Fe(III) and it contains calcium ion.

The specification teaches that in order to grow the cells used in the presently described studies, the formulations of serum-free defined medium employed are specific optimizations, modifications, or necessary changes of earlier media that have been described (p. 89, para 0355). The formulations presented permit dissection of growth into its individual parts caused by different stimulators. Said serum-free medium provides a tool for the assessment of growth inhibitors isolated from horse serum whose actions are reversed by sex-steroid hormones. Thus the serum-free media raises the hope for the provision of a new insight that could help to clarify the mechanisms involved in the control of breast, prostatic and other mucosal cancers under conditions not previously available (p. 95, para 0374).

The specification teaches that it is widely believed that the phenol red indicator in tissue culture medium acts as a weak estrogen and at the concentration in standard culture media, it was believed to stimulate ER+ cell growth nearly as well a natural estrogens (para 0318). However, using the defined steroid free nutrient medium taught in the specification it was possible to determine that the concentration of phenol red contaminants in a standard culture medium today is not sufficient to cause estrogenic effects. Demonstration of sex hormone mitogenic effects in culture depends upon conditions that maximize the effects of a serum-borne inhibitor as described in the Examples. When the effects of the inhibitor are optimized, the presence or absence of phenol red makes no everyday

difference to the demonstration of estrogen mitogenic effects with several target cell types from diverse species (p. 74, para 0319). The specification specifically teaches the composition of serum free defined media and serum-free media variations in TABLE 7 and on pages 93-94. Further the specification teaches that the media described in TABLE 7 were optimized for the specific cell types designated. Additionally, they were optimized to permit direct comparison of the growth properties of ER+ and AR+ steroid hormone sensitive tumor cell lines to their ER- and AR- steroid hormone insensitive counterparts. This careful optimization was done originally to study rat mammary tumor cells. The specification discloses that previously, there was a defined medium for the prostate cancer cell line PC3, but that this medium was evaluated and did not support LNCaP prostate cancer cell line growth. Although other have reported "serum-free' media that was stated to be effective with the LNCaP cell lines, the problem was that this medium was not serum-free nor was it defined. Under those conditions, an accurate analysis of hormonal and growth factor effects cannot be done satisfactorily (p. 98). The new serum-free defined medium serves as part of a model system for identifying physiologically relevant new molecules. When completely serum-free defined conditions were employed in the past, the effects of estrogens were either marginal or insignificant (p. 104, para 0399).

One cannot extrapolate the teaching of the specification to the scope of the claims because the specification clearly teaches that the "new" serum-free defined medium is a critical element of the claimed invention. The specification clearly teaches that in order to grow the cells used in the presently described studies, the formulations of serum-free defined medium employed are specific optimizations, modifications, or necessary changes of earlier media that have been described and

that the formulations presented permit dissection of growth into its individual parts caused by different stimulators and exemplifies the inability of standard culture media to permit dissection of growth into its individual parts caused by different stimulators. In particular, the specification teaches that when completely serum-free defined conditions were employed in the past, the effects of estrogens were either marginal or insignificant. Specifically, the new media were carefully optimized to permit direct comparison of growth properties of ER+ and AR+ steroid hormone sensitive tumor cell lines and their ER- and AR- steroid hormone insensitive counterparts. Further, the specification teaches that although others have reported "serum-free" media that was stated to be effective, for example, for growing the LNCaP prostate cancer cell line, the problem with this medium was that it was not in fact serum-free, nor was it defined. Under those conditions, an accurate analysis of hormonal and growth factor effects **cannot** (emphasis added)be done satisfactorily. The new serum-free defined medium serves as part of a model system for identifying physiologically relevant new molecules. Although the specification specifically states that the formulations of serum-free defined medium employed are specific optimizations, modifications, or necessary changes of earlier media that have been described and that the formulations presented permit dissection (for the first time) of growth into its individual parts caused by different stimulators, the specification provides only a single example of a medium that will function as claimed with defined variations depending upon the requirements of the selected cells to be cultured but does not teach how to successfully use the claimed invention with any other medium. This is critical because the specification specifically teaches that when completely serum-free defined conditions were employed in the past, the effects of estrogens were either

marginal or insignificant and that in the absence of the defined medium, an accurate analysis of hormonal and growth factor effects **cannot** (emphasis added) be done satisfactorily. It is clear that in years of research, no other media/defined media has been found to be effective to function as claimed. The single example of a steroid hormone-free nutrient medium, modifiable for specific cell lines, does not provide guidance for how one would make any other medium that produces conditions that that maximize the effects of a serum-borne inhibitor as described in the Examples. Although experimentation to provide optimization is not considered undue, the specification does not teach the "necessary" changes of earlier media that are required so that one could make a medium that would function as claimed. Thus, the specification does not provide guidance on how one would make the claimed invention. For the above reasons, it appears that undue experimentation would be required to practice the claimed invention in the absence of the defined medium taught in the specification.

Claim Rejections - 35 USC § 102

15. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

16. Claims 34-42, 95, 109-112 are rejected under 35 U.S.C. § 102(b) as being anticipated by US Patent No. 5,135,849.

The claims are drawn to an *in vitro* method for detecting steroid hormone-like cancer cell growth stimulation by a substance of interest comprising

maintaining a predetermined population of steroid hormone-responsive cells in a steroid hormone-free basal nutrient medium substantially devoid of unbound Fe(III) and containing calcium ion comprising a quantity of immunoglobulin inhibitor sufficient to inhibit cell growth in the absence of an inhibition-reversing amount of said steroid hormone, said cells being steroid hormone dependent for proliferation in vivo, adding said substance of interest to said cells, incubating and determining the cell population wherein a measurable increase in said cell population indicates a steroid hormone-like cell growth stimulating effect by said substance of interest (claim 34), comprising maintaining serum-free assay conditions (claim 35), comprising adding steroid-hormone depleted serum to said nutrient medium (claim 36), further comprising obtaining non-heat inactivated serum containing said immunoglobulin inhibitors (claim 37), wherein said immunoglobulin inhibitor comprises at least one secretory immunoglobulin chosen fro the group consisting of dimeric/polymeric IgA, polymeric IgM and IgG (claim 38), wherein at least one secretor immunoglobulin is chosen from the group consisting of IgG1 and IgG2 (claim 39), wherein at least one secretory immunoglobulin is IgG1kappa claim 40), wherein said substance contains or is suspected of containing proteolytic activity, the method comprising selecting an immunoglobulin inhibitor that resists protease degradation (claim 41), wherein said selected immunoglobulin inhibitor comprises IgA2 (claim 42), said method using a population of estrogen-responsive cells, whereby an estrogen-like cell growth stimulating effect by said substance is detected and whereby an estrogenic substance is detected (claim 95), whereby said steroid hormone free nutrient medium comprises no more than about 1 micromolar unbound Fe(III) (claim 109), wherein the medium comprises a Fe(111) chelating agent (claim 110), wherein the

medium comprises a cell attachment promoting protein (claim 111),whereby said medium contains about 1-50 mM calcium ion (claim 112).

It is noted that in the absence of a definition for inactivated serum, it is assumed for examination purposes that inactivated serum means serum that has had active ingredients, such coagulation factors, removed.

US Patent No 5,135,849 specifically teaches an *in vitro* method for detecting steroid hormone-like cancer cell growth stimulation by a substance of interest (wherein said substances produces, emulates or mimics the effect of naturally occurring endogenous steroid hormone [col 8, lines 12-15]) comprising maintaining a predetermined population of steroid hormone-responsive LNCaP cells in a steroid hormone-free basal nutrient medium substantially devoid of unbound Fe(III) and containing calcium ion (Dulbecco's Eagle's Minimal Essential Medium, claim 6 wherein said medium is substantially devoid of Fe(III), that is approximately .25 micromolar, and comprises approximately 1.25 mM calcium as per Invitrogen catalogue) wherein the medium comprises a quantity of inhibitor of androgen dependent cell proliferation which is endogenous to the serum of humans or animals sufficient to inhibit cell growth in the absence of an inhibition-reversing amount of said steroid hormone, said cells being steroid hormone dependent for proliferation *in vivo*, adding said substance of interest to said cells, incubating and determining the cell population wherein a measurable increase in said cell population indicates a steroid hormone-like cell growth stimulating effect by said substance of interest, (claim 1) that is the overcoming of the inhibition of androgen stimulated cell proliferation, wherein functional equivalents of charcoal dextran stripped human serum are used in the assay, that is the inhibitor is semi-purified (cols 19-20) and thus the maintaining medium is in

fact serum free, comprising adding steroid-hormone depleted serum to said nutrient medium (claim 7), further comprising obtaining non-heat inactivated serum containing said immunoglobulin inhibitors (see column 20 wherein heat inactivated serum, that is serum which has had the coagulation factors removed, is obtained). The specification further teaches that the serum born inhibitor is found in the sera of male and female humans, bovines, horses, pigs, dogs, rats and mice and that it is heat stable (col 10, lines 61-70). Although the prior art specification does not specifically teach that the inhibitor comprises immunoglobulins, secretory immunoglobulin chosen from the group consisting of dimeric/polymeric IgA, polymeric IgM and IgG, IgG1 and IgG2, IgG1 kappa, IgA2, given that the instantly claimed immunoglobulin inhibitors are isolated from serum wherein the prior art reference specifically teaches that the serum borne inhibitor is found in male and female humans, bovines, horses, pigs, dogs, rats and mice, it is clear that at least a subset of the inhibitor components include all of the claimed immunoglobulin inhibitors since these are ubiquitously found in serum and thus at least a subset of the inhibitor components of the prior art reference appears to be the same as the instantly claimed immunoglobulin inhibitors. The office does not have the facilities and resources to provide the factual evidence needed in order to establish that the product of the prior art does not possess the same material, structural and functional characteristics of the claimed product. In the absence of evidence to the contrary, the burden is on the applicant to prove that the claimed product is different from that taught by the prior art and to establish patentable differences. See In re Best 562F.2d 1252, 195 USPQ 430 (CCPA 1977) and Ex parte Gray 10 USPQ 2d 1922 (PTO Bd. Pat. App. & Int. 1989). Further, the method of the prior art comprises the same method steps as claimed in the instant

invention, that is, assaying for steroid-hormone like effects of substances of interest comprising assaying the same population of cells with a medium comprising a serum borne inhibitor found in male and female humans, bovines, pigs, dogs, rats, mice and horses from which the instantly claimed immunoglobulins were isolated, thus the claimed method is anticipated because the serum borne inhibitor inherently comprises the claimed immunoglobulin inhibitors. See Ex parte Novitski 26 USPQ 1389 (BPAI 1993). It is noted that, because of structural differences, IgA2 lacks sites required for proteolysis (see (Chintalacharuvu K R and Morrison S L (1996) J Immunol 157, 3443-3449 cited in the specification) thus would clearly resist protease degradation.

In addition, the specification teaches that the LNCaP cells are estrogen-responsive, see Figure 2 and demonstrates the growth stimulating effect of an estrogenic substance wherein the estrogenic substance is detected (see Table 1). Finally, upon addition of the charcoal dextran stripped serum, the assay medium comprises the proteins of the serum wherein serum in well known to contain both apotransferrin, an effective Fe (111) chelator (see Eby et al (1992, Anal Biochem, 203,317-325 cited in the specification) and fibronectin, a cell attachment promoting protein (see Tr. J of Medical Sciences, 1998, 28:383-387, see in particular the abstract and page 383, col 1, page 386, para bridging cols 1 and 2). All the limitations of the claims are met.

It is noted that the specification admits on the record that patents have been issued drawn to an uncharacterized serum mediator that is steroid hormone reversible in steroid responsive cells and points specifically to US Patent No. 5,135,849. Given the broadly written claims, the US Patent reads on the claimed invention for the reasons set forth above. The rejection can be obviated by

amending the claims to recite, for example, "comprising a steroid hormone reversible inhibitor, wherein said inhibitor consists of an immunoglobulin or a combination of immunoglobulins".

Claim Objections

17. Claim 37 is objected to because although the claim is drawn to the method of claim 34 further comprising obtaining non-heat inactivated serum containing said immunoglobulin inhibitors. However, there are no method steps recited for the obtaining non-heat inactivated serum. There appears to be a typographical error, does Applicant intend to claim the method of claim 34 further comprising adding non-heat inactivated serum containing said immunoglobulin inhibitors? Appropriate correction is required.

18. Claims 41 is objected to because there is no antecedent basis for the recitation of "the method comprising selecting an immunoglobulin" in claim 34 from which it depends. The objection could be obviated by amending the claim, for example to recite "the method further comprising selecting an immunoglobulin."

19. Claims 34-43, 109-113 and 114 are objected to as being dependent upon non-elected claim 45. The rejection can be obviated by amending claim 34 to recite all of the limitations of claim 45 and deleting reference to claim 45.

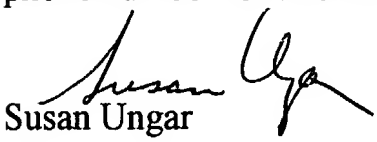
20. Please note that the none of the references disclosed on the IDS forms submitted 6/7/04 or 4/4/03 have been considered because the references were not found in the file. If the references were indeed submitted, Examiner would appreciate the resubmission of these references so that they can be considered.

21. No claims allowed.

22. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Susan Ungar, PhD whose telephone number is

(571) 272-0837. The examiner can normally be reached on Monday through Friday from 7:30am to 4pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeffrey Siew, can be reached at (571) 272-0787. The fax phone number for this Art Unit is (571) 273-8300.



Susan Ungar
Primary Patent Examiner
February 9, 2005

Form PTO-1449 (Modified)

INFORMATION DISCLOSURE STATEMENT BY APPLICANT
(Use several sheets if necessary)

Atty. Docket No.	Serial No.
1944-00201	09/852,958
Applicant	
David A. Sirbasku	
Filing Date	**Group**
05/10/2001	1623

REFERENCE DESIGNATION U.S. PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE
SM	AA	5,135,849	08/04/92	Soto et al.	435	29	
	AB	5,075,425	12/24/91	Kotitschke et al.	530	387	
	AC	4,859,585	08/22/89	Sonnenschein et al.	435	29	
	AD	4,849,508	07/18,89	Magnin et al.	530	387	

FOREIGN PATENT DOCUMENTS

		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUB-CLASS	Translation YES	NO
SM	AE	EP 0702960A	27/03/96	Europe	A61K 39	395		
	AF	WO 00/06723	10/02/00	PCT	C12N	15/12		
	AG	WO 95/09011	06/04/95	PCT	A61K	48/00		
	AH	WO 91/16061	31/10/91	PCT	A61K	35/14		

OTHER ART (Including Author, Title, Date, Pertinent Pages, Etc.)

SM	AI	Partial International Search in PCT/US 01/15183, Applicant David A. Sirbasku, International Filing Date 10/05/2001, Date of Mailing 27/08/2002, pages 4
	AJ	Partial International Search in PCT/US 01/15171, Applicant David A. Sirbasku, International Filing Date 10/05/2001, Date of Mailing 29/08/2002, pages 3
	AK	JP Parisot et al., "Altered Expression of the IGF-1 Receptor in a Tamoxifen-Resistant Human Breast Cancer Cell Line," BRITISH JOURNAL OF CANCER, vol. 79, no. 5-6, pp. 693-700, 1999
	AL	David Danielpour et al., "Growth of MTW9/PL2 Estrogen-Responsive Rat Mammary Tumor Cells in Hormonally Defined Serum-Free Media," IN VITRO CELL. DEV. BIOL., vol. 24, no. 1, pp. 42-52, January 1988
	AM	Research Diagnostics Inc., 'Online! 10 January 2000 (2000-01-10), XP002207863, Retrieved from the Internet: <URL:http://www.researchd.com/rdiabs/igref.ser.htm> 'retrieved on 2002-07-29! The whole document, page 1
	AN	Jorge E. Moreno-Cuevas et al., "Estrogen Mitogenic Action. I. Demonstration of Estrogen-Dependent MTW9/PL2 Carcinogen-Induced Rat Mammary Tumor Cell Growth in Serum-Supplemented Culture and Technical Implications," IN VITRO CELL. DEV. BIOL.—ANIMAL, vol. 36, no. 7, pp. 410-427, July-August 2000
	AO	David A. Sirbasku et al., "Estrogen Mitogenic Action. II. Negative Regulation of the Steroid Hormone-Responsive Growth of Cell Lines Derived From Human and Rodent Target Tissue Tumors and Conceptual Implications," IN VITRO CELL. DEV. BIOL.—ANIMAL, vol. 36, no. 7, pp. 428-446, July-August 2000

1 SM 2/24/05

Form PTO-1449 (Modified)	Atty. Docket No. 1944-00201	Serial No. 09/852,958
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use several sheets if necessary)	Applicant David A. Sirbasku	
	Filing Date 05/10/2001	Group 1623

continuation,

OTHER ART (Including Author, Title, Date, Pertinent Pages, Etc.)

SM	AP	Helenice Gobbi et al., "Transforming Growth Factor-β and Breast Cancer Risk in Women With Mammary Epithelial Hyperplasia," JOURNAL OF THE NATIONAL CANCER INSTITUTE, vol. 91, no. 24, pp. 2096-2101, December 15, 1999,
	AQ	Ruth Sager, "Expression Genetics in Cancer: Shifting the Focus from DNA to RNA," PROC. NATL. ACAD. SCI. USA, vol. 94, no. 3, pp. 952-955, February 1997
	AR	Sanford D. Markowitz et al., "Tumor Suppressor Activity of the TGF-β Pathway in Human Cancers," CYTOKINE & GROWTH FACTOR REVIEWS, vol. 7, no. 1, pp. 99-102, 1996

EXAMINER	DATE CONSIDERED
	2/24/05

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP §609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to the applicant.

Notice of References Cited	Application/Control No. 09/852,958	Applicant(s)/Patent Under Reexamination SIRBASKU, DAVID A.	
	Examiner Susan Ungar	Art Unit 1642	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-			
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Yildirim (Tr. J. Med. Sci., 1998, 28:383-387
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

2/24/05

Tr. J. of Medical Sciences
28 (1998) 383-387

Ali YILDIRIM

The Role of Serum on the Adhesion of Cultured Chinese Hamster Lung (CHL) Cells

Received: September 20, 1996

Atatürk University, Department of Chemical Education, K. Karabekir Education Faculty, Erzurum-Turkey

Abstract: The roles of serum on the initial attachment and adhesion strength of CHL cells, which is gained following initial attachment, spreading and growth, on tissue culture polystyrene dish were determined by using a convergent Microflow chamber. In the presence of 10% fetal calf serum 98%±2% of cells were attaching within 2 hours at 37°C. While only 74%±3% of cells were attaching during the same period of incubation in serum free medium. The critical shear stresses of detachment of CHL cells were 3.01±0.25 Nm^{-2}, 5.38±0.55 Nm^{-2}, 7.22±0.54 Nm^{-2}, 9.47±0.43 Nm^{-2}, and 9.54±1.15 Nm^{-2} in the presence of 0%, 0.05%, 0.1%, 1%, and 5% fetal calf serum respectively. In addition the effect of serum origins on the adhesion strength of this cell was determined. The the critical shear stresses of detachment of CHL cells were1.73±0.80 Nm^{-2}, 11.6±0.66 Nm^{-2}, and 10.96±0.73 Nm^{-2} in the presence of 10% of horse serum, new born calf serum, and fetal calf serum respectively. These results suggest that 1% serum concentration will be sufficient for cells to gain the maximum possible adhesion strength.

Key Words: CHL Cells; Adhesion strength; serum proteins.

Introduction

Cell substrate adhesion is a multistep process including initial cell contact to the substratum, attachment, spreading and growth (1-3). An understanding of animal cell adhesion may be important in controlling practical problems such as the control of cell growth on a biocompatible substrate, the proper anchoring of connective tissue on to metal bone prostheses and the prevention of attachment of blood cells to vascular prostheses. The investigation of all these areas requires the measurement of cell adhesion (4).

It is generally believed that the growth of almost all types of mammalian cells in culture require the presence of added serum in the culture medium (5,6). Serum is an extremely complex mixture of many molecules. There might be components of serum which are yet undefined. However, the major functions of serum can be broadly defined: these arettchment and spreading, nutrition, stimulation, and protection (7). Recently, rapid progress has been made in the identification and characterization of the serum proteins involved in cell adhesion such as fibronectin, vitronectin, laminin, thrompospondin (8,9). There are also many poorly studied proteins (adhesive proteins) found to mediate cell adhesion (10.11). In conrast, some serum proteins interfere with cell attachment, anti adhesive proteins, (12,13). For example, it has been shown that both α-1-antitrypin and albumin reduce adhesiveness of BHK cells (14) while immunoglobulin G (IgG) inhibits hepatocyte adhesion (15).

However, most of above serum-cell adhesion studies deal qualitatively with only initial cell attachment which is the first step in cell adhesion. Whereas, in this study the role of serum in cell adhesion, that is gained following initial attachment, spreading, and growh (3,6), was determined by using the Microflow chamber which has been developed recently (16). Hence, the relationships between the strength of cell binding on a tissue culture dish and origins of serum or concentrations of fetal calf serum in culture medium have been studied.

Materials and Methods

Cell Culture: Cultured Chinese Hamster Lung (CHL) cells were obtained from Flow Laboratories UK. CHL cells were maintained in minimum essential Eagle Medium (EMEM) with Earls salts supplemented with 20mM N-2-hydroxyethyl piperazine-N-ethanesulphonic acid (HEPES) buffer, 10% (v/v) fetal calf serum, 200 IU penicillin/ml, 200mg streptomycin/ml, 2mM glutamine and 2% non essential amino acids. Cultures were incubated in an atmosphere of 5% Co_2/air (17).

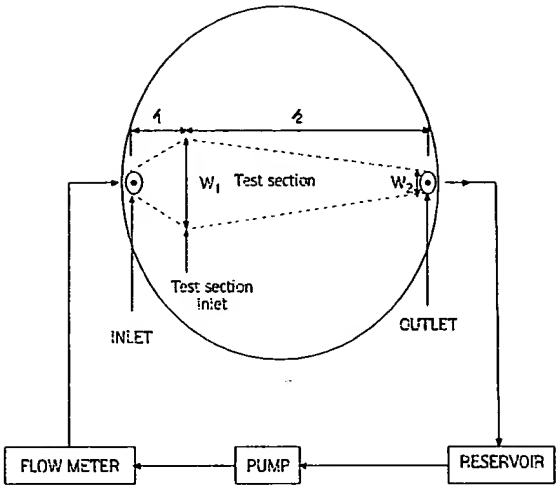


Figure 1. General Arrangement of Microflow Chamber and the Attached Apparatus (l_1:20mm, l_2:47mm, w_1:20mm, w_2:7mm)

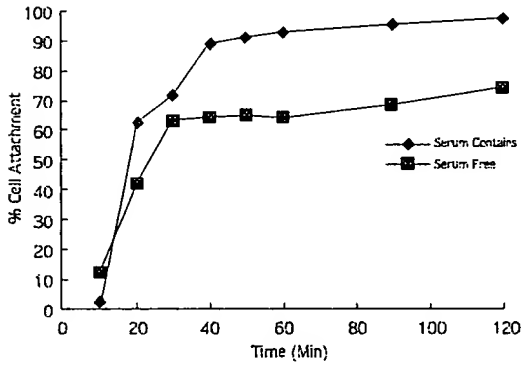


Figure 2. CHL cells attachment in the presence of 10% fetal calf serum or in the absence of serum.
Attachment studies were performed on 35mm tissue culture grade plastic dishes. 2ml of cell suspension contain totally $1x10^6$ cells were added into these dishes. After incubation at 37°C for indicated period the number of attached and non attached cells were determined.

Attachment Assay: A Sub confluent monolayer of cells was trypsinized with 0.05% w/v typsin in ethylenediamintetraacetic acid-phosphate buffered salina (EDTA-PBS) buffer and the action of this proteolytic enzyme wes stopped by using serum containing culture medium. The density of the cells was determined by means of a Neubauer hemocytometer. Then a stock cell suspension that contained $5x10^5$ cells/ml was prepared by

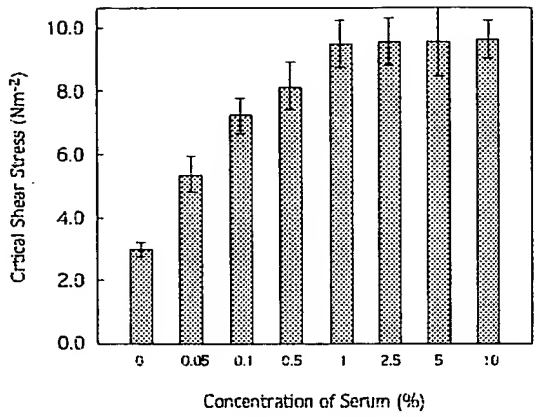


Figure 3. CHL Cells Adhesion Strength at the Various Concentrations of Fetal Calf Serum.
Sub confluent CHL cells weresubcultured and maintained in the culture medium that was supplemented with 0% to 10% (v/v) serum. The adhesion strength of the CHL cells growing in these media is measured in terms of the critical shear stress (c.s.s) of detachment.

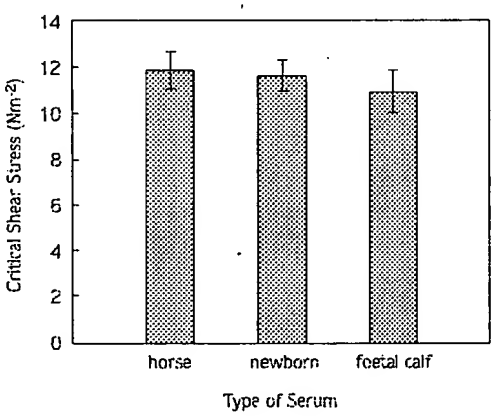


Figure 4. The Effect of Origin of Serum on CHL Cells Adhesion Strength.
CHL cells were seeded in the medium supplemented with 10% horse serum, or new born calf serum, or fetal calf serum on to plastic substratum. The adhesion strength of CHL Cells growing in these mediums is measured in terms of the critical shear stress (c.s.s) of detachment.
(P=0.02 between horse serum and fetal calf serum; P=0.04 between new born calf serum and fetal calf serum).

diluting the above cell suspension with growth medium. Subsequently, 2mls of the latter cell suspension were distributed onto 35 mm round tissue culture dishes which then were incubated at 37°C. Finally, after the incubation period, the culturemedium was transferred into a tube and all unattached cells were removed by washing dish with serum free medium. Any cell not removed by series of these gentle washes were considered to be attached. The numbers of attached and non- attached cells were counted in an haemocytometer. Experiments 5 times repeated in each of which 2 determinations were made. Without serum, attachment assay was performed as above except that trypsin was inhibited by soybean trypsin inhibitor (Sigma) and cells were cultured in serum free medium.

Detachment Assay: The 20 ml cell suspension, that was prepared from subconfluent cells as described above, was poured into a 100 mm tissue culture grade plastic dish. The cells were allowed to grow for 24 hours in 5% (V/V) CO_2/air atmophere at 37°C. Finally, the adhesion strength of these cells were measured by inserting this cell growing substratum on the Microflow chamber and after passing the running medium (serum free medium) over test substratum at defined flow rates. After 10 minutes of running; the distance from the beginning of test section to a point at which cells start to detach (critical distance) was measured by a ruler. By inserting this value and flow rate in equation:

$$\tau = \frac{13.15 \times V}{73 \cdot L}$$

where

τ= Shear stress (N/m^2),

V= Flow rate (ml/s),

L= Critical distance (mm),

the shear stress of detachment of cell was determined (16). Experiments were repeated 4 times in each of which 10 determinations were made.

The Microflow chamber, basically, is a round shaped aluminum casting 100 mm diameter and coated with nylon.. A convergent channel is incorporated into this chamber. The width of this convergence at the beginning of this channel is 20mm tapering to 7mm at the end of the channel (figure 1). The depth of channel is constant (1mm). The cell culture dish forms the lid of the chamber. The dish and chamber is clamped tight and running medium at a convenient flow rate (determined by several trials) is pumped into chamber. Hence channel is convergent, the velocity of fluid passing through channel increase from inlet to outlet and hydrodynamic shear stress on cell growing substratum increase as well. At a certain critical point the surface shear stress becomes sufficiently large to cause the detachment of the cells. The distance from test section inlet to this detachment point called critical distance and is used in equation above to determined call adhesion strength (16).

Results

The amount of atached cells in 10 minute of incubation was higher in the absence of serum then those in 10% serum containing medium. However, after 60 minutes of incubation 93%±3% cells were attaching in the presence of serum while 64%±4% of cells were attaching during the same period in the absence of serum (figure 2). Over 60 minutes of incubation, there were slight increases in the amount of attached cells. Nevertheless, these were not significant in both cases. Hence after 2 hours of incubation 97%±2% and 74%±3% of cells were attching in the presence of 10% serum and in the absence of serum respectively.

Cell adhesion strength increased with increasing serum concentrations up to 1% of serum. While above 1% increasing the serum concentration did not have any noticeable effect upon the strength of CHL cells. In the absence of serum the adhesion strength of CHL cells was very low; the c.s.s of detachment being 3.01±0.25 Nm^{-2}. When the serum concentration was increased, the critical shear force required to detach cells from tissue culture dish increased as well. That is the values for the critical shear stress of detachment were 7.22±0.54 Nm^{-2}, 8.14±0.74 Nm^{-2}, and 9.47±0.43 Nm^{-2} in the presence of 0.1%, 0.5%, and 1% fetal calf serum in the culture medium respectively. Thus it is clear that upto a concentration of 1% serum, cell adhesion strength was serum-dosage dependent. Increasing serum concentration above 1% did not affect cell adhesion significantly. Hence the c.s.s of detachments were 9.54±1.15 Nm^{-2} and 9.60±0.75 Nm^{-2} in the presence of 5%, and 10% serum respectively. Although there were slight differences between these values, these were not statistically significant. For instance there was no significant difference in the c.s.s of detachments even between 1% and 10% of serum (P=0.67). (Statistical calculations were done by using Minitab software version 8.2)

The CHL cell adhesion strength was highest in 10%horse serum and lowest in the fetal calf serum. The actual values for the c.s.s. of detachment were 11.73±0.80 Nm^{-2} and 10.96±0.73 Nm^{-2} in horse serum

and fetal calf serum respectively (P=0.02) and it was 11.66±0.66 Nm^{-2} in new born calf serum (P=0.04, between new born calf serum and fetal calf serum respectively), figure 4. These results indicate that there is no significant effect of the origin of serum in cell adhesion strength, as far as above serum origins and CHL cells are concerned.

Discussion

The role of serum on the cell attachment, which is only initial step in cell adhesion, have previously been studied (18). However, there are so far no reports that indicate the role of serum on the cell adhesion process. In the present study, the effect of concentrations and origins of serum on the CHL cells adhesion strength, that is ganied following initial contact, attachment, spreading and growth (1,2,3), were determined quantitatively by Microflow chamber. In addition the attachment rates of CHL cells in the presence of 10% fetal calf serum or in the absence of serum were determined.

There was a lag phase in the CHL cells attachment curve in the serum presence, while in the absence of serum there was no lag phase. This difference could be due to that in the presence of cells do not directly adhere to the surface, instead they adhere via cell surface receptors. Whereas in the absence of serum there are no preadsorbed protenis on the substratum and simply cells adsorb to the surface non specifically (19). Thus in the first case cell attachment involves different subsequent steps such as, receptor-ligand binding, signaling, and reorganizations of other molecules that are involved in cell adhesion (20,21,22). This will of course take time and therefore as soon as the cells touch the surface they adsorb to it (23). In fact the duration of the lag period in the presence of serum increased as the incubation temperature decreased. Whereas in the absence of serum there was no lag phase even at 4°C (data is not shown).

In the absence of serum cell adhesion is performed by direct interaction between cell surface molecules and substratum (23,24). Hence, there is no further process for the cell to strengthen its adhesion. While in the presence of serum, cell adhesion is mediated by surface adsorbed serum proteins. If serum is considered to be the only source of proteins for cell adhesion, it appears that fetal calf serum, even at a concentration of 1%, provided sufficient amounts of adhesive proteins for CHL cells to gain their maximum possible adhesion strength. Fibronectin and vitronectin are two major cell adhesion proteins in serum (10,25). However, some of the proteins in serum affect the adsorptive properties of the adhesive proteins adversely. For example, albumin, and α-2-macroglobulin, serum anti adhesive proteins, reduce fibronectin adsorption (12). It was reported that the adsorption of fibronectin decreases at a serum concentration of 2% or above while the vitronectin adsorption increases (19,25). The above reports, coupled with CHL cell adhesion strength-serum concentration studies make it possible to suggest that at low serum concentrations CHL cells gain their adhesive strength mainly through fibronectin, while at high serum concentrations fibronectin has a limited role and at such concentrations CHL cells will attain their adhesion strength through vitronectin. Consequently, it is possible to suggest that, since fibronectin receptors and vitronectin receptors are integrin receptors (26) both of these receptors might exert the same effect on the cell, as far as the strength of cell adhesion is concerned.

There was no significant effect of origins of serum on cell adhesion strength, as far as the serum origins and CHL cells used in this study are concerned. In fact it has been reported that there is no detectable difference between horse serum fibronectin and calf serum fibronectin, as far as structure and functions are concerned (27). Moreover, it has been previously determined that six different animals vitronectins, which play a major role in the cell adhesion at 10% serum in the culture medium (19), did not significantly vary in their effect on BHK cells adhesion (28) These findings support results obtained in the present study, although there are as yet no reports that indicate the relationship between the origin of the sera and cell adhesion strength. In this work it was possible to show quantitatively that origin of serum has no significant effect on CHL cell adhesion strength.

Perhaps it sholud be mentioned here that the effect of serum in cell adhesion is not limited to the adhesion proteins. There are other serum components that might also have a role in cell adhesion. These components may include molecules such as tranferrin and growth hormones (29,30,31).

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